Relationship Between Insulin Resistance and β-Cell Dysfunction in Subphenotypes of Prediabetes and Type 2 Diabetes

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Context: There is little overlap between diabetes diagnosed by glycated hemoglobin (HbA_{1c}) and blood glucose, and it is unclear which pathophysiological defects are captured when using HbA_{1c} for diagnosis.

Objective: We examined and compared the relationship between insulin sensitivity and β-cell function in different subphenotypes of prediabetes and type 2 diabetes (T2D).

Design, Setting, and Participants: A cross-sectional analysis of the Danish ADDITION-PRO study was performed (n = 1713). Participants without known diabetes were classified into subgroups of prediabetes and T2D based on fasting or 2-hour glucose criteria or HbA_{1c}. Insulin sensitivity and insulin release were determined from glucose and insulin concentrations during the oral glucose tolerance test, and disposition indices were calculated.

Results: Individuals with prediabetes or T2D diagnosed by fasting glucose had lower absolute insulin release ($P \leq .01$) and higher insulin sensitivity in response to glucose intake ($P \leq .01$) but a similar disposition index ($P \geq .36$), compared with individuals with elevated 2-hour glucose concentrations. Individuals with HbA_{1c}-defined T2D or prediabetes had a mixture of the pathophysiological defects observed in the glucose-defined subgroups, and individuals with normoglycemia by HbA_{1c} had worse pathophysiological abnormalities than individuals with normoglycemia by the glucose criteria.

Conclusions: On average, the diagnostic HbA_{1c} criteria for diabetes and prediabetes identified individuals with a mixture of the pathophysiological characteristics found when using the glucose criteria, but the diversity and pathophysiology captured by the oral glucose tolerance test cannot be captured when applying the more simple HbA_{1c} criteria. Whether the disease progression and prognosis will differ in individuals diagnosed by fasting glucose, 2-hour glucose, or HbA_{1c} should be examined in longitudinal studies. **(*J Clin Endocrinol Metab* 100: 707–716, 2015)**

The somewhat controversial term prediabetes covers a number of high-risk states for developing type 2 diabetes (T2D) and cardiovascular disease (CVD) (1, 2). The most commonly studied subphenotypes of prediabetes are impaired fasting glycaemia (IFG) and impaired glucose tolerance (IGT), both of which can appear in isolation

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Abbreviations: BIGTT-AIR, index of acute insulin response; BMI, body mass index; CVD, cardiovascular disease; DI, disposition index; F-DM, diabetes by elevated fasting glucose only; F-2h-DM, diabetes by both elevated fasting and 2-hour glucose concentrations; HbA_{1c}, glycated hemoglobin; 2h-DM, diabetes by elevated 2-hour glucose only; HOMA-IS, homeostasis model assessment of insulin sensitivity; IFG, impaired fasting glycemia; IFG+IGT, combination of IFG and IGT; IGT, impaired glucose tolerance; i-IFG, isolated impaired fasting glycemia; i-IGT, isolated impaired glucose tolerance; ISI_{0-120}, insulin sensitivity index; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; T2D, type 2 diabetes; VAT, visceral abdominal adipose tissue.

(i-IFG or i-IGT) or in combination (IFG+IGT). These phenotypes are identified by measuring plasma glucose concentrations in the fasting state and after an oral glucose tolerance test (OGTT).

In some individuals, T2D develops as a consequence of early β-cell dysfunction; in others, the development of insulin resistance precedes defects in the pancreatic β-cells (3–5). These findings underscore that T2D is not a single disease entity but rather multiple diseases or phenotypes with different origins and disease developments. Thus, it is likely that the heterogeneity observed in the prediabetic stages is still present when fasting and 2-hour glucose levels increase into the diabetic range. Part of this heterogeneity may be explained by differences in overall obesity or body fat distribution because obesity and especially the visceral abdominal adipose tissue (VAT) are associated with insulin resistance (6, 7).

In addition to fasting and 2-hour glucose concentrations, glycated hemoglobin (HbA_{1c}) is now recommended for diagnosis of prediabetes and T2D (8, 9). HbA_{1c} in the prediabetic and diabetic range is associated with increased CVD morbidity and mortality (10–12), and because HbA_{1c} does not require a fasting sample and has considerably lower variability than the glucose measures, it is often the preferred test. However, it is not clear whether the pathophysiological defects present in the distinct fasting and 2-hour glucose-derived prediabetic and diabetic subphenotypes are captured in the new HbA_{1c}-based prediabetic and diabetic phenotypes. More epidemiological studies focusing on the heterogeneity of prediabetes and T2D will increase our understanding of the complexity of T2D as well as the implications of changing diagnostic criteria. In combination with smaller metabolic studies, large-scale epidemiological studies can also contribute to developing targeted strategies for prevention and treatment of T2D.

The aim of this study was to examine and compare the relationship between insulin sensitivity and β-cell function in different subphenotypes of prediabetes and screen-detected T2D diagnosed by fasting glucose, 2-hour glucose, and HbA_{1c} criteria. Moreover, we aimed to examine whether the pathophysiological differences in subphenotypes of prediabetes and T2D could be explained by differences in overall and abdominal obesity.

Research Design and Methods

Study population

The ADDITION-PRO study is a longitudinal risk-stratified cohort study of individuals at high risk for developing T2D. After participation in a population-based step-wise screening program in Danish general practice between 2001 and 2006 (13), 16 136 eligible individuals were identified. All individuals with impaired glucose regulation at screening and a random subsample of individuals at lower diabetes risk were invited to attend a follow-up health examination in 2009–2011. Of these participants, 2082 (50% of invited), mainly of white ethnicity (98%), attended (14).

The study was approved by the Ethical Committee of the Central Denmark Region (journal number 20080229) and was conducted in accordance with the Helsinki Declaration. All participants provided written informed consent before participating in the study.

Study procedures 2009–2011

After an overnight fast of 8 or more hours, a standard 75-g OGTT was given to all participants without known diabetes, and a physical examination was performed. Blood samples were drawn at 0, 30, and 120 minutes for the assessment of plasma glucose and serum insulin concentrations.

Information on age and sex was obtained from the unique Danish civil registration number. Body weight was measured in light indoor clothing without shoes to the nearest 0.1 kg with a Tanita body composition analyzer, and height was measured to the nearest millimeter using a fixed rigid stadiometre (Seca; Medical Scales and Measuring Systems). Clothes were estimated to weigh 0.5 kg, which was deducted from the total body weight, and body mass index (BMI) was calculated. Waist circumference was measured with an unstretchable tape measure to the nearest millimeter at the midpoint between the lower costal margin and the anterior superior iliac crest. Abdominal fat distribution was assessed by ultrasonography according to a validated protocol on a subset of participants (n = 1463 with complete data) with the participant in the supine position (Logiq9 ultrasound machine; GE Healthcare). The transducer was placed on the abdomen at the point at which the xiphoid line crosses the waist circumference. The measurement was performed at the end of a quiet expiration with minimum pressure of the transducer on the skin. The VAT was assessed by measuring the intraperitoneal distance with a 4°C (1.5–4.5 MHz) transducer placed in the longitudinal position. The distance from the peritoneum to the spine was measured twice, and the average of the two measurements was used (15). The within- and between-sonographer coefficients of variation for the VAT were 4.0% or less. The ADDITION-PRO study is described in detail elsewhere (14).

Classification of subphenotypes of prediabetes and T2D

We classified all study participants according to the OGTT [World Health Organization 2006 criteria (16)] as having normal glucose tolerance (NGT), intermediate hyperglycemia (i-IFG, i-IGT, IFG+IGT) or screen-detected T2D by fasting glucose only (F-DM), 2-hour glucose only (2h-DM) or both fasting and 2-hour glucose (F-2h-DM). In addition, all participants were classified according to their HbA_{1c} levels as having normal HbA_{1c}, high-risk (prediabetic) HbA_{1c}, or screen-detected T2D (9) (Supplemental Figure 1). The overlap between the different diagnostic criteria for prediabetes and T2D is shown in Supplemental Figure 2.

Biochemical measures

All biochemical measures were performed at the Steno Diabetes Center (Gentofte, Denmark). Plasma glucose was determined using the Hitachi 912 system (Roche Diagnostics) or the

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Vitros 5600 system (Ortho Clinical Diagnostics). Based on validation analyses performed at the laboratory at the Steno Diabetes Center, all Vitros values (71% of all) were converted to Hitachi values using the following equation: adjusted value = (original glucose value + 0.2637)/0.983. There was a high correlation between original and converted values ($r^2 = 0.9974$). Serum insulin was measured by an immunoassay (AutoDELFIA; PerkinElmer). HbA_{1c} was measured by HPLC (TOSOH G7).

Calculations

Using plasma glucose and serum insulin levels from the OGTT, we calculated the insulin sensitivity index (ISI_{0-120}) (17), reflecting insulin sensitivity after oral glucose intake, and the homeostasis model assessment of insulin sensitivity (HOMA-IS) (18), reflecting insulin sensitivity in the fasting state. As a measure of absolute β-cell function, the index of acute insulin response (BIGTT-AIR) was calculated (19). BIGTT-AIR reasonably well reflects first-phase insulin release as measured by an iv glucose tolerance test in individuals with prediabetes (20). Because the amount of insulin secreted from the β-cells depends on the plasma glucose levels and the degree of insulin resistance, we also estimated two different oral disposition indices (DI): one multiplying BIGTT-AIR with ISI_{0-120} (DI_{OGTT}) and one multiplying BIGTT-AIR with HOMA-IS ($DI_{fasting}$). The rationale for calculating both of these indices is that insulin resistance measured in the fasting state and after glucose intake reflects different mechanisms. Insulin resistance in the fasting state is mainly related to the liver, whereas the peripheral tissues play a more predominant role for insulin resistance measured during an OGTT; both features are relevant when estimating the DI (21).

Statistical analysis

The present analysis is based on data collected at the follow-up examination (cross-sectional analysis). Participants with known diabetes (n = 336), those fasting less than 8 hours prior to the health examination (n = 19), and those who could not be classified due to missing information were excluded (n = 14), leaving 1713 individuals for analysis (only 1463 for analyses including VAT).

To examine differences in characteristics between glycemic groups, we performed an overall ANOVA, and if significant, post hoc *t* tests were used to study pairwise differences. The first model was adjusted for age and sex; the next included further adjustment for BMI or VAT, height, and waist circumference. The ISI_{0-120}, HOMA-IS, BIGTT-AIR, and disposition indices were logarithmically transformed before the analysis to fulfill the assumption of normality of the residuals. Statistical analyses were performed in SAS version 9.2 (SAS Institute). A two-sided 5% level of significance was adjusted for multiple testing with the method of Benjamini and Hochberg (22) in all pair-wise comparisons regarding insulin sensitivity and β-cell function.

Results

Characteristics of the study population

Using the OGTT criteria, more men than women had i-IFG and T2D ($P \leq .047$ for pair-wise differences; Table 1). Individuals with i-IGT were slightly older than the other groups ($P \leq .043$) except the 2h-DM group ($P = .179$). BMI, VAT, and waist circumference were higher in individuals with T2D and IFG+IGT than in those with NGT ($P < .001$), and the F-2h-DM group had the highest BMI ($P \leq .003$), waist circumference ($P \leq .009$), and VAT ($P \leq .028$) among all groups.

Table 1. Characteristics of the 1713 Study Participants in the ADDITION-PRO Cohort According to Normal Glucose Tolerance and Subtypes of Pre-diabetes and Diabetes Defined by the Glucose and HbA_{1c} Criteria

Characteristics	n	Men, %	Age, y	BMI, kg/m²	Waist Circumference, cm	VAT, cm (n = 1463)
OGTT Criteria						
NGT	899	47.9 (44.6–51.3)	65.8 (7.2)	26.1 (4.1)	30.9 (8.2)	7.3 (2.3)
i-IFG	336	63.4 (58.0–68.6)[a]	66.3 (6.7)	27.8 (4.3)[a]	31.9 (8.1)[a]	8.5 (2.6)[a]
i-IGT	134	47.8 (39.1–56.6)[b]	68.3 (6.0)[a,b]	27.3 (4.3)[a]	33.3 (7.8)[a]	8.2 (2.7)[a]
IFG+IGT	162	54.3 (46.3–62.2)	66.3 (6.3)[c]	29.2 (4.5)[a,b,c]	35.0 (7.9)[a,b,c]	8.8 (2.8)[a]
F-DM	91	64.8 (54.1–74.6)[a,c]	66.4 (6.5)[c]	28.7 (5.5)[a]	32.9 (8.5)[a,c]	9.3 (3.2)[a,b,c]
2h-DM	48	64.6 (49.5–77.8)[a,c]	66.7 (6.4)	29.5 (4.0)[a,,b,c]	34.3 (8.4)[a,b,c]	9.9 (3.2)[a,b,c,d]
F-2h-DM	43	72.1 (56.3–84.7)[a,c,d]	64.8 (6.2)[c]	32.2 (5.6)[a,b,c,d,e,f]	36.2 (8.9) [a,b,c,d,e,f]	11.3 (2.8) [a,b,c,d,e,f]
Overall *P* value		<.001	.010	<.001	<.001	<.001
HbA_{1c} criteria						
HbA_{1c} < 6.0% (<42 mmol/mol)	1335	53.0 (50.3–55.7)	66.0 (7.0)	26.7 (4.2)	31.5 (8.2)	7.7 (2.5)
HbA_{1c} 6.0–6.4% (42–46 mmol/mol)	324	54.3 (48.7–59.8)	67.4 (6.3)[g]	28.4 (4.9)[g]	33.3 (8.3)[g]	8.9 (3.1)[g]
HbA_{1c} ≥ 6.5% (≥48 mmol/mol)	54	61.1 (46.9–74.1)	64.3 (6.9)[h]	31.7 (5.4)[g,h]	36.7 (9.0)[g,h]	10.7 (3.2)[g,h]
Overall *P* value		.478	<.001	<.001	<.001	<.001

Data are percentages (95% confidence interval) or means (SD).

[a] OGTT criteria, $P < .05$ vs NGT. [b] OGTT criteria, $P < .05$ vs i-IFG. [c] OGTT criteria, $P < .05$ vs i-IGT. [d] OGTT criteria, $P < .05$ vs IFG+IGT. [e] OGTT criteria, $P < .05$ vs F-DM. [f] OGTT criteria, $P < .05$ vs 2 h-DM. [g] HbA_{1c} criteria, $P < .05$ vs HbA_{1c} less than 6.0%.

[h] HbA_{1c} criteria, $P < .05$ vs HbA_{1c} 6.0%–6.4%.

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By the use of the HbA_{1c} criteria, the proportion of men and women did not differ between groups, but individuals with prediabetes were significantly older than those with T2D or normal HbA_{1c} ($P \leq .002$; Table 1). BMI, VAT, and waist circumference were highest in individuals with T2D ($P < .001$ for all) but also were higher in individuals with prediabetic HbA_{1c} compared with the group with normal HbA_{1c} ($P < .001$).

Insulin sensitivity and absolute insulin secretion

After adjustment for age, sex, and multiple testing, insulin sensitivity after oral glucose intake (ISI_{0-120}) was not statistically significantly different between the i-IGT and F-DM group ($P = .093$) but differed between all other groups with the i-IGT and 2h-DM groups having lower ISIO-120 than the i-IFG and F-DM groups, respectively ($P \leq .012$ for all; Figure 1A). The 2h-DM, IFG+IGT, and F-DM groups did not differ with regard to insulin sensitivity in the fasting state (HOMA-IS, $P \geq .103$; Figure 1B), but there was a tendency of a difference between the i-IFG and i-IGT groups ($P = .057$). All other groups differed from each other ($P \leq .019$) with the F-2h-DM group having the lowest HOMA-IS (Figure 1B). In terms of absolute β-cell function, BIGTT-AIR was significantly lower in individuals with i-IFG ($P < .001$) and IFG+IGT ($P < .001$) but was higher in the i-IGT group ($P < .001$) than in the NGT group (Figure 1, A and B). The diabetic groups with F-DM and F-2h-DM did not differ with regard to BIGTT-AIR ($P = .505$), and those with 2h-DM had BIGTT-AIR levels comparable with the NGT ($P = .164$) and i-IGT ($P = .497$) groups (Figure 1, A and B). All other comparisons of BIGTT-AIR were statistically significant ($P \leq .002$ for all).

In general, the group with normal HbA_{1c} seemed to have lower levels of absolute insulin secretion and insulin sensitivity compared with those with NGT (Figure 1, C and D). Within the HbA_{1c} groups, those with prediabetes had lower BIGTT-AIR than those with normal HbA_{1c} ($P < .001$) but higher than those with T2D ($P = .007$).

Disposition indices

There was a step-wise decline in DI_{OGTT} from NGT to prediabetes and T2D ($P < .001$ for all; Figure 2A). However, DI_{OGTT} was not significantly different when comparing i-IFG with i-IGT ($P = .599$) and F-DM with 2h-DM ($P = .361$). All other pair-wise differences in DI_{OGTT} were statistically significant ($P \leq .003$ for all). $DI_{fasting}$ was lower in i-IFG than in i-IGT ($P < .001$) and in F-DM than in 2h-DM ($P < .001$). In contrast, $DI_{fasting}$ was not lower in individuals with IFG+IGT ($P = .102$) or 2h-DM ($P = .214$) than in those with i-IFG; but all other differences in $DI_{fasting}$ were statistically significant ($P \leq .034$; Figure 2B).

Compared with individuals with NGT, people with $HbA_{1c} < 6.0\%$ tended to have lower disposition indices (Figure 2, A and B). Both DI_{OGTT} and $DI_{fasting}$ differed significantly among all three HbA_{1c} groups ($P \leq .001$).

Role of obesity

Figure 3 shows percentage differences in the above pathophysiological features in the different OGTT and HbA_{1c} defined subphenotypes of prediabetes and T2D in relation to those with normal glucose regulation before and after adjustment for BMI, age, and sex. With the adjustment for BMI, the following differences became insignificant: BIGTT-AIR between i-IGT and NGT ($P = .069$), HOMA-IS between F-2h-DM and F-DM ($P = .191$), ISI_{0-120} between IFG+IGT and i-IGT ($P = .124$) and between F-2h-DM and 2h-DM ($P = .111$). All other differences remained statistically significant after adjustment for BMI (and multiple comparisons). By adjustment for VAT, height, and waist circumference the same differences as adjustment for BMI became insignificant. In addition, the differences in HOMA-IS between IFG+IGT and i-IFG ($P = .177$) and between 2h-DM and i-IGT ($P = .068$) disappeared.

A schematic overview of the differences in absolute early insulin release, insulin sensitivity, and disposition indices between groups is presented in Table 2.

Discussion

This study highlights that T2D is not a single disease entity but rather multiple subdiseases or subphenotypes characterized by different underlying pathophysiological mechanisms, starting in the early prediabetic states. Our findings also suggest that the differences in β-cell function and overall insulin sensitivity between subgroups of individuals with prediabetes and type 2 diabetes are not explained by overall and visceral fat. In addition, we showed that the newly implemented diagnostic HbA_{1c} criteria on average identify prediabetic and diabetic individuals with a mixture of pathophysiological characteristics compared with those found by the glucose criteria. However, the normoglycemic individuals defined by HbA_{1c} have slightly worse insulin resistance and β-cell function than those with normoglycaemia by the OGTT.

Subphenotypes of prediabetes

Based on the OGTT criteria, we identified three subgroups of prediabetes and three subgroups of T2D, all with different underlying pathophysiology. Although we found large variation within each group, the mean differences between subphenotypes of prediabetes are in alignment with previous observations. By use of detailed gold

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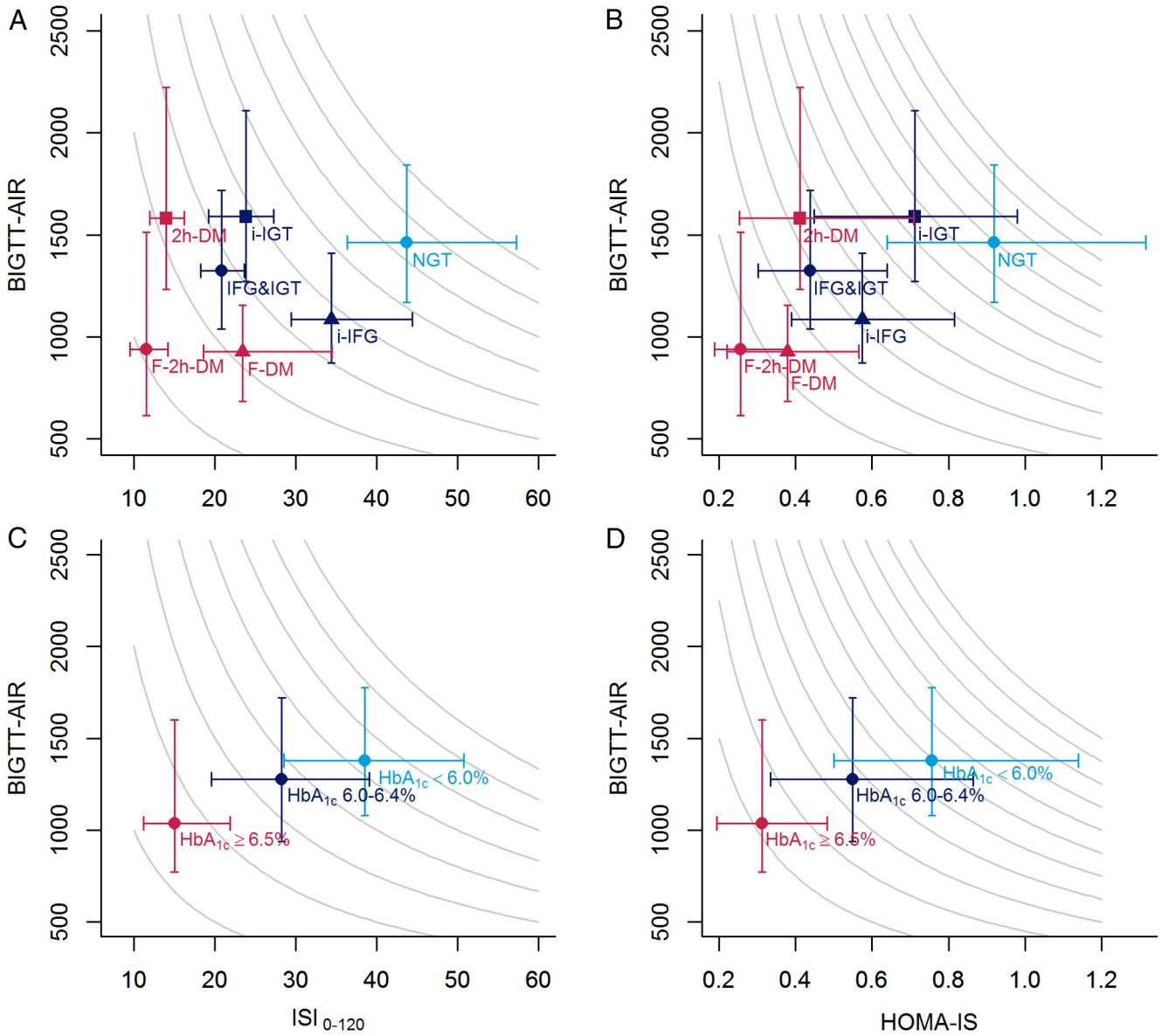

Figure 1. BIGTT-AIR as a function of ISI_{0-120} (panels A and C) and HOMA-IS (panels B and D) in individuals with different subphenotypes of prediabetes and type 2 diabetes based on the glucose (panels A and B) or HbA_{1c} criteria (panels C and D). The thin gray lines show different levels of the disposition index. Data are medians (interquartile range).

standard methods, it has been shown that insulin resistance in the liver is a characteristic of people with i-IFG, whereas insulin resistance in the peripheral tissues is an important feature of i-IGT (23–26). Our surrogate markers of insulin sensitivity in the fasting state (mainly liver) and during the OGTT (mainly peripheral tissues) support this notion.

Individuals with i-IFG had reduced early-phase insulin release compared with individuals with i-IGT, which is also in accordance with previous findings (23, 24). It has previously been demonstrated that obese individuals with i-IFG have a 40% deficit in relative β-cell volume compared with obese individuals with normoglycemia (27). This observation suggests that the β-cell failure observed in individuals with i-IFG represents an early process in the development of T2D, which is not likely to be secondary to hyperglycemia or insulin resistance. Whether the reduction in β-cell function in i-IFG is caused by genetic factors, chronic low-grade inflammation, amyloid deposition, or other factors (28–30) warrant further studies.

In terms of relative β-cell function, DI_{OGTT} was comparable between the i-IFG and i-IGT groups, whereas $DI_{fasting}$ was lower in i-IFG than in i-IGT, potentially demonstrating different relative contributions of the liver and the skeletal muscles in the control of fasting vs post-OGTT glucose regulation (21, 31, 32). The IFG+IGT group had a combination of the defects observed in the isolated IFG and IGT groups but with disposition indices close to the levels observed in F-DM and 2h-DM, sup-

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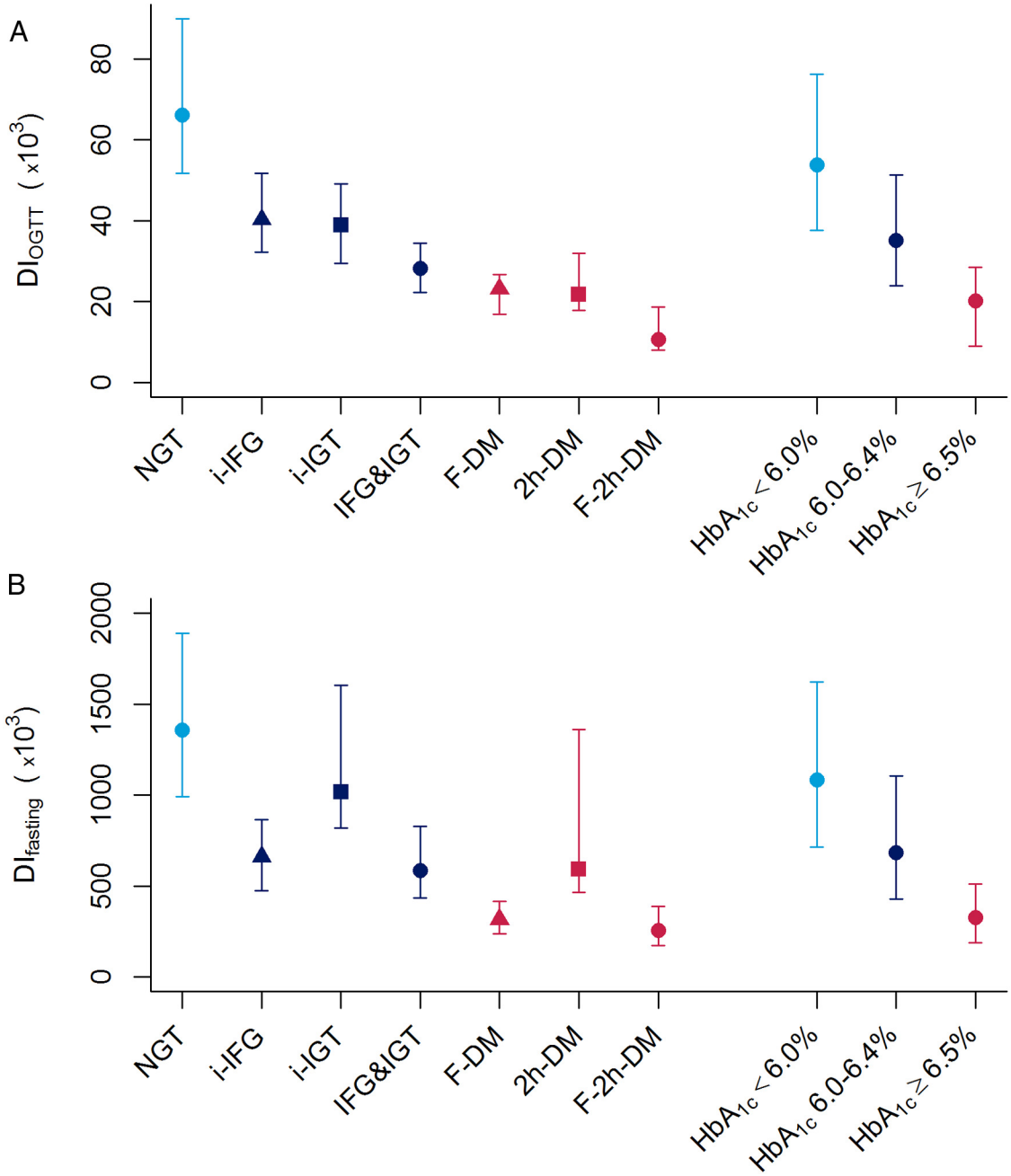

Figure 2. Disposition indices calculated from BIGTT-AIR and ISI_{0-120} (DI_{OGTT}) (panel A) and from BIGTT-AIR and HOMA-IS ($DI_{fasting}$) (panel B) in different groups of prediabetes and type 2 diabetes defined by the glucose and HbA_{1c} criteria. Data are medians (interquartile range).

porting progressive β-cell failure when moving from i-IFG to IFG+IGT (33).

Prediabetic individuals defined by HbA_{1c} had pathophysiological defects resembling a mixture of the three prediabetic groups defined by the OGTT. That HbA_{1c} reflects an average of the pathophysiological defects captured by the OGTT-defined groups of prediabetes has also been found in two Italian studies (34, 35).

Subphenotypes of type 2 diabetes

We hypothesized that the pathophysiological drivers of elevated fasting and 2-hour glucose concentrations in the prediabetic states may continue to operate in the diabetic range. Therefore, we subdivided patients with screen-detected T2D into subgroups based on the fasting and 2-hour plasma glucose levels.

Compared with individuals with i-IGT, diabetic individuals with isolated elevated 2-hour glucose concentrations (ie, 2h-DM) had the same level of absolute early insulin release but significantly lower insulin sensitivity in both the fasting and glucose-stimulated state, resulting in lower disposition indices. Despite the cross-sectional nature of these results, they indicate that progression from i-IGT to 2h-DM is characterized by development of insu-

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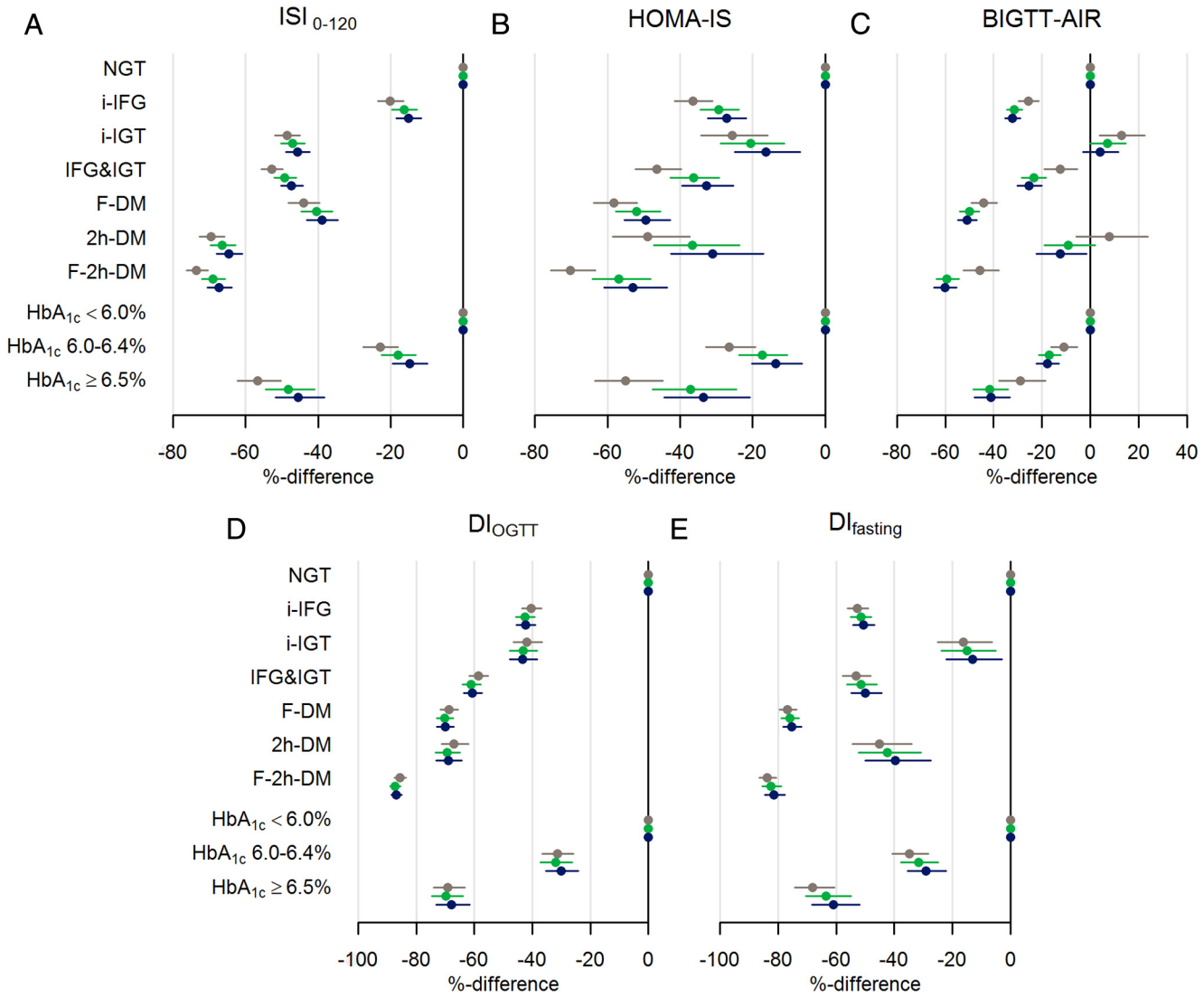

Figure 3. Relative difference in ISI_{0-120} (A), HOMA-IS (B), BIGTT-AIR (C), DI_{OGTT} (D), and $DI_{fasting}$ (E) in individuals with different subphenotypes of prediabetes and type 2 diabetes compared with NGT as well as in individuals with high-risk HbA_{1c} or screen-detected diabetes by HbA_{1c} in relation to normal HbA_{1c}. Gray lines, Adjusted for age and sex. Green lines, Adjusted for age, sex, and BMI. Blue lines, Adjusted for age, sex, VAT, height, and waist circumference (n = 1463). Data are medians (interquartile range).

lin resistance and a lack of ability to sufficiently compensate by increasing insulin secretion. Recently we showed in a longitudinal cohort of British individuals that the 2h-DM phenotype was preceded by a steep increase in 2-hour insulin concentration, a decline in insulin sensitivity, and a stable relative β-cell function (3), supporting our observations from the present study.

In contrast to the 2h-DM phenotype, F-DM was characterized by significantly reduced β-cell function as compared with i-IFG individuals, both in absolute terms and in relation to insulin resistance (DI_{OGTT} and $DI_{fasting}$). Individuals with F-DM also had a significant reduction in insulin sensitivity as compared with individuals with i-IFG. Chinese individuals with F-DM were also characterized by reduced β-cell function as compared with individuals with i-IFG, whereas whole-body insulin sensitivity did not differ between people with i-IFG and F-DM (5). In the longitudinal Whitehall II study, individuals diagnosed with F-DM had impaired β-cell function up to 18 years before their T2D diagnosis (3), suggesting that a reduction in the insulin secretory capacity precedes the development of peripheral insulin resistance in individuals with isolated fasting hyperglycaemia. However, more prospective studies are needed to confirm this hypothesis.

As expected, the F-2h-DM phenotype was characterized by worse pathophysiological defects than the groups diagnosed with isolated fasting or 2-hour hyperglycaemia. These results are in accordance with other studies (3, 5). However, most people will progress to F-2h-DM from either F-DM, 2h-DM, or IFG+IGT and the underlying pathophysiological defects leading to F-2h-DM may

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Table 2. Overview of the Defects in Insulin Sensitivity and β-Cell Function Observed in the Different Subgroups of Prediabetes and T2D

	Absolute Early Insulin Release	Insulin Sensitivity (Glucose-Stimulated State)	Insulin Sensitivity (Fasting State)	Relative β-Cell Function (Glucose Stimulated State)	Relative β-Cell Function (Fasting State)
OGTT definition					
NGT	Ref.	Ref.	Ref.	Ref.	Ref.
i-IFG	↓ ↓	↔/↓	↓ ↓	↓	↓ ↓
i-IGT	↔/↑	↓ ↓	↔/↓	↓	↓
IFG+IGT	↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓
F-DM	↓ ↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓ ↓
2h-DM	↔	↓ ↓ ↓	↓ ↓	↓ ↓	↓ ↓
F-2h-DM	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓
HbA_{1c} definition					
$HbA_{1c} < 6.0\%$	↔	↔	↔	↔	↔
HbA_{1c} 6.0%–6.4%	↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓
$HbA_{1c} \geq 6.5\%$	↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓

↔, unchanged; ↓, mildly decreased; ↓ ↓, moderately decreased; ↓ ↓ ↓, highly decreased; ↑, mildly increased; Ref, Reference.

therefore differ, depending on how far in the diabetes progression the individual is.

The diversity found within the diabetes subgroups captured by the glucose criteria cannot be reflected when using the HbA_{1c} definition of diabetes because this is only one category. In general, patients with HbA_{1c}-diagnosed diabetes had insulin resistance and β-cell dysfunction in the same low range as patients diagnosed by F-DM and F-2h-DM, suggesting that the cut point for HbA_{1c} of 6.5% identifies individuals in direct need for therapies to correct disturbances in beta cell function.

Role of obesity

Because obesity and mainly abdominal fat is an important driver of insulin resistance (6, 7), we hypothesized that part of the differences in insulin sensitivity observed between the prediabetic and diabetic subphenotypes were attributed differences in overall and/or abdominal obesity. BMI or VAT did not explain the differences in ISI_{0-120} between the i-IFG vs i-IGT or F-DM vs 2h-DM groups, but differences in HOMA-IS between groups were largely explained by differences in overall and particularly abdominal visceral obesity. This observation indicates a close link between fat depots in the abdomen and glucose regulation in the fasting state, potentially mediated by adipokines (36). Of interest, adjustment for overall or abdominal visceral obesity also explained the excessive early insulin release in the i-IGT group compared with i-IFG. However, it did not change the differences in disposition indices between groups, indicating that the effect of obesity is accounted for by taking insulin resistance into account when estimating in vivo β-cell function.

Fasting glucose, OGTT, or HbA_{1c} for diagnosis?

The availability and practical feasibility of the different diagnostic tests should drive the decision of which test to use. Yet our findings show that diagnosis of prediabetes or T2D based on fasting glucose, 2-hour glucose, or HbA_{1c} will identify people with a different underlying pathophysiology. On average, HbA_{1c} identifies individuals with both insulin resistance and β-cell function, but HbA_{1c} does not reflect the heterogeneity of prediabetes and T2D captured by the OGTT and therefore cannot stand alone if reversal of the underlying pathophysiology is a treatment goal (32).

Despite the normal absolute insulin secretion in individuals with 2h-DM, they have the same excess risk of all-cause and CVD mortality as individuals diagnosed with T2D based on fasting glucose or HbA_{1c} (37). Only part of the individuals with 2h-DM (~42%) will be captured by the new HbA_{1c} criterion for diabetes (38), and because of the limited use of the OGTT in clinical practice, many of these high-risk individuals will remain undiagnosed.

Strengths and limitations

The main strength of this cross-sectional study is the large number of study participants from whom we have measurements of circulating glucose and insulin concentrations during OGTTs as well as detailed measures of anthropometry. Because of the large study population, we used surrogate measures of insulin sensitivity, early insulin release, and β-cell function. Because fasting and 2-hour glucose concentrations were included in the calculations of insulin sensitivity and β-cell function, they partly overlap with the classification of prediabetes and T2D by the glucose criteria. However, in the calculations of ISI_{0-120} and BIGTT-AIR, also fasting and 2-hour insulin concentrations as well as information on body weight was included, limiting the risk of circular conclusions. Moreover, the pathophysiological defects in prediabetic individuals observed using surrogate measures of insulin sensitivity and early insulin release correspond well with

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previous findings in smaller studies using the gold standard clamp technique as well as an iv glucose tolerance test (24). This suggests that relatively simple surrogate measures can be used to estimate pathophysiological defects in high-risk individuals. How to implement knowledge on individual pathophysiology in the prevention and treatment of T2D still needs to be determined. Because of the relatively large day-to-day variation in fasting and especially 2-hour plasma glucose levels (39) and thereby a potential risk of misclassification, repeated measurements are necessary before universal or population-specific cut points for determining, for example, insulin resistance should be used on an individual level.

The sampling of participants for the ADDITION-PRO study was predominantly based on glucose tolerance status as measured by an OGTT, and not by HbA_{1c}, at a step-wise screening 5–7 years before the ADDITION-PRO examination (14). This selection of participants means that the likelihood of being classified with prediabetes or diabetes by the OGTT at follow-up was larger than the likelihood of being classified by HbA_{1c}. Therefore, the distribution of participants according to the different diagnostic criteria is not representative for the general Danish population. There is no reason to believe that the differences found between groups should not apply to the general population in Denmark, but our findings need confirmation in other European and non-European cohorts and ultimately in longitudinal studies in which the pathophysiology underlying progression from one glucose tolerance state to another can be determined.

Conclusion and perspectives

In conclusion, the relative contributions of insulin resistance and defective insulin release differ widely between the prediabetic and diabetic subgroups diagnosed by fasting vs 2-hour glucose concentrations. Individuals diagnosed with prediabetes or T2D by elevated fasting plasma glucose levels had lower absolute insulin release and higher overall insulin sensitivity than those diagnosed by 2-hour glucose concentrations, although their overall β-cell function (ie, DI) was comparable. Overall and abdominal obesity partly explained this diversity.

On average, the diagnostic HbA_{1c} criteria for diabetes and prediabetes identified individuals with a mixture of the pathophysiological characteristics found when using plasma glucose criteria, but the diversity identified by the glucose criteria is not captured when applying the more simple HbA_{1c} criteria. Our findings confirm that T2D is not a single disease entity but rather multiple subdiseases with different characteristics. There is a need for longitudinal studies examining whether disease progression and prognosis will differ in individuals diagnosed by fasting glucose, 2-hour glucose, or HbA_{1c}. Moreover, randomized controlled trials should clarify whether a treatment targeting the different phenotypes will prevent the progression of prediabetes or T2D and reverse β-cell dysfunction in individuals with early insulin-secretory defects.

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References

1. **Perreault L, Færch K.** Approaching pre-diabetes. *J Diabetes Complications*. 2014;28(2):226–233.
2. **Færch K, Johansen NB, Vistisen D, Jørgensen ME.** Cardiovascular

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risk stratification and management in pre-diabetes. *Curr Diab Rep*. 2014;14:493.
3. **Færch K, Witte DR, Tabak AG, et al.** Trajectories of cardiometabolic risk factors before diagnosis of three subtypes of type 2 diabetes: a post-hoc analysis of the longitudinal Whitehall II cohort study. *Lancet Diabetes Endocrinol*. 2013;1(1):43–51.
4. **Tabák AG, Jokela M, Akbaraly TN, Brunner EJ, Kivimäki M, Witte DR.** Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. *Lancet*. 2009;373(9682):2215–2221.
5. **Qian L, Xu L, Wang X, et al.** Early insulin secretion failure leads to diabetes in Chinese subjects with impaired glucose regulation. *Diabetes Metab Res Rev*. 2009;25(2):144–149.
6. **Preis SR, Massaro JM, Robins SJ, et al.** Abdominal subcutaneous and visceral adipose tissue and insulin resistance in the Framingham heart study. *Obesity*. 2010;18(11):2191–2198.
7. **Wander PL, Boyko EJ, Leonetti DL, McNeely MJ, Kahn SE, Fujimoto WY.** Change in visceral adiposity independently predicts a greater risk of developing type 2 diabetes over 10 years in Japanese Americans. *Diabetes Care*. 2013;36(2):289–293.
8. **American Diabetes Association.** Standards of medical care in diabetes—2013. *Diabetes Care*. 2014;37(suppl 1):S14–S80.
9. **World Health Organization.** *Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus. Abreviated Report of a WHO Consultation*. Geneva: WHO Press, editor; 2011:1–25.
10. **Selvin E, Steffes MW, Zhu H, et al.** Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. *N Engl J Med*. 2010; 362(9):800–811.
11. **Barr EL, Boyko EJ, Zimmet PZ, Wolfe R, Tonkin AM, Shaw JE.** Continuous relationships between non-diabetic hyperglycaemia and both cardiovascular disease and all-cause mortality: the Australian Diabetes, Obesity, and Lifestyle (AusDiab) study. *Diabetologia*. 2009;52(3):415–424.
12. **Khaw KT, Wareham N, Luben R, et al.** Glycated haemoglobin, diabetes, and mortality in men in Norfolk cohort of european prospective investigation of cancer and nutrition (EPIC-Norfolk). *BMJ*. 2001;322(7277):15–18.
13. **Lauritzen T, Griffin S, Borch-Johnsen K, Wareham NJ, Wolffenbuttel BH, Rutten G.** The ADDITION study: proposed trial of the cost-effectiveness of an intensive multifactorial intervention on morbidity and mortality among people with type 2 diabetes detected by screening. *Int J Obes Relat Metab Disord*. 2000;24(suppl 3):S6–S11.
14. **Johansen NB, Hansen AL, Jensen TM, et al.** Protocol for ADDITION-PRO: a longitudinal cohort study of the cardiovascular experience of individuals at high risk for diabetes recruited from Danish primary care. *BMC Public Health*. 2012;12:1078.
15. **Stolk RP, Wink O, Zelissen PM, Meijer R, van Gils AP, Grobbee DE.** Validity and reproducibility of ultrasonography for the measurement of intra-abdominal adipose tissue. *Int J Obes*. 2001;25(9): 1346–1351.
16. **World Health Organization and International Diabetes Federation.** *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia: Report of a WHO/IDF Consultation*. Geneva: World Health Organization; 2006.
17. **Gutt M, Davis CL, Spitzer SB, et al.** Validation of the insulin sensitivity index (ISI0,120): comparison with other measures. *Diabetes Res Clin Pract*. 2000;47(3):177–184.
18. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.** Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412–419.
19. **Hansen T, Drivsholm T, Urhammer SA, et al.** The BIGTT test: a novel test for simultaneous measurement of pancreatic β-cell function, insulin sensitivity, and glucose tolerance. *Diabetes Care*. 2007; 30(2):257–262.
20. **Færch K, Pacini G, Nolan JJ, Hansen T, Tura A, Vistisen D.** Impact of glucose tolerance status, sex, and body size on glucose absorption patterns during OGTTs. *Diabetes Care*. 2013;36(11):3691–3697.
21. **Færch K, Brøns C, Alibegovic AC, Vaag A.** The disposition index: adjustment for peripheral vs. hepatic insulin sensitivity? *J Physiol*. 2010;588(5):759–764.
22. **Benjamini Y, Hochberg Y.** Controlling the false discovery rate—a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol*. 1995;57(1):289–300.
23. **Abdul-Ghani MA, Jenkinson CP, Richardson DK, Tripathy D, DeFronzo RA.** Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance—results from the Veterans Administration genetic epidemiology study. *Diabetes*. 2006;55(5):1430–1435.
24. **Færch K, Vaag A, Holst J, Glümer C, Pedersen O, Borch-Johnsen K.** Impaired fasting glycaemia vs impaired glucose tolerance: similar impairment of pancreatic α and β cell function but differential roles of incretin hormones and insulin action. *Diabetologia*. 2008;51(5): 853–861.
25. **Meyer C, Szoke E, Pimenta W, et al.** Different mechanisms for impaired fasting glucose and impaired postprandial glucose tolerance in humans. *Diabetes Care*. 2006;29(8):1909–1914.
26. **Tripathy D, Almgren P, Tuomi T, Groop L.** Contribution of insulin-stimulated glucose uptake and basal hepatic insulin sensitivity to surrogate measures of insulin sensitivity. *Diabetes Care*. 2004; 27(9):2204–2210.
27. **Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC.** β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. *Diabetes*. 2003;52(1):102–110.
28. **Kolb H, Mandrup-Poulsen T.** The global diabetes epidemic as a consequence of lifestyle-induced low-grade inflammation. *Diabetologia*. 2010;53(1):10–20.
29. **Jurgens CA, Toukatly MN, Fligner CL, et al.** β-Cell loss and β-cell apoptosis in human type 2 diabetes are related to islet amyloid deposition. *Am J Pathol*. 2011;178(6):2632–2640.
30. **Halban PA, Polonsky KS, Bowden DW, et al.** β-Cell failure in type 2 diabetes: postulated mechanisms and prospects for prevention and treatment. *J Clin Endocrinol Metab*. 2014;99(6):1983–1992.
31. **O'Rahilly S, Hattersley A, Vaag A, Gray H.** Insulin resistance as the major cause of impaired glucose tolerance: a self-fulfilling prophesy? *Lancet*. 1994;344(8922):585–589.
32. **DeFronzo RA.** Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes*. 2009;58(4):773–795.
33. **Perreault L, Bergman BC, Playdon MC, la-Man C, Cobelli C, Eckel RH.** Impaired fasting glucose with or without impaired glucose tolerance: progressive or parallel states of prediabetes? *Am J Physiol Endocrinol Metab*. 2008;295(2):E428–E435.
34. **Bianchi C, Miccoli R, Bonadonna RC, et al.** Pathogenetic mechanisms and cardiovascular risk: differences between HbA1c and oral glucose tolerance test for the diagnosis of glucose tolerance. *Diabetes Care*. 2012;35(12):2607–2612.
35. **Calanna S, Scicali R, Di Pino A, et al.** α- and β-Cell abnormalities in haemoglobin A1c-defined prediabetes and type 2 diabetes. *Acta Diabetol*. 2014;51(4):567–575.
36. **Tonjes A, Fasshauer M, Kratzsch J, Stumvoll M, Bluher M.** Adipokine pattern in subjects with impaired fasting glucose and impaired glucose tolerance in comparison to normal glucose tolerance and diabetes. *PLoS One*. 2010;5(11):e13911.
37. **Silbernagel G, Sourij H, Grammer TB, et al.** Isolated post-challenge hyperglycaemia predicts increased cardiovascular mortality. *Atherosclerosis*. 2012;225(1):194–199.
38. **Xu Y, Wang L, He J.** Prevalence and control of diabetes in chinese adults. *JAMA*. 2013;310(9):948–959.
39. **Mooy JM, Grootenhuis PA, de Vries H, et al.** Intra-individual variation of glucose, specific insulin and proinsulin concentrations measured by two oral glucose tolerance tests in a general Caucasian population: the Hoorn Study. *Diabetologia*. 1996;39(3):298–305.

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